Derlin-1 Is Overexpressed on the Tumor Cell Surface and Enables Antibody-Mediated Tumor Targeting Therapy

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Abstract

**Purpose:** Tumor targeting therapy is one of the most promising strategies for anticancer treatment. Derlin-1 has been reported to participate in misfolded protein dislocation and integrates into the endoplasmic reticulum (ER) membrane to survey for such protein aggregates. We elucidate herein that Derlin-1 can leak to the plasmalemma from the ER in tumor cells and may have clinical application as a novel cancer target in the hope of developing a new tumor targeting therapy.

**Experimental Design:** The cell surface expression of Derlin-1 was shown by immunofluorescence analysis of nonpermeabilized cells and Western blotting of fractional proteins of tumor cells. Derlin-1 expression in cancerous tissues was also shown by immunohistochemistry. Biodistribution analysis and γ-scintigraphic imaging were done using125I-labeled Derlin-1-targeting antibody in isogenic mice models. Finally, tumor-bearing mice were treated by the anti-Derlin-1 polyclonal antibody and monoclonal antibodies.

**Results:** Derlin-1 was expressed on various tumor cell surfaces and adopted a homodimer conformation. Robust cytoplasmic and membrane expression of Derlin-1 was detected in various types of human cancers tissues but was not correlated with any clinicopathologic features of pancreatic cancer. Derlin-1 directed antibodies specifically targeted to colon tumors and significantly suppress tumor growth in isogenic mice.

**Conclusions:** These preclinical data show that Derlin-1 protein is a functional molecular target expressed on the tumor cell surface and is a candidate therapeutic target that may be translated into clinical applications.

The membrane compartment of tumor cells contains many receptors that are required for cell survival, proliferation, and metastasis. Therapeutic approach by targeting these membrane proteins with molecular drugs, such as monoclonal antibodies, has been recently relatively developed to impressive result (1–3). For optimized targeting of tumors, antigen targets should have high expression in the tumor, restricted normal tissue expression, little or no soluble form, and accessibility to circulating antibody (4).

Derlin-1 is reported to participate in the dislocation of misfolded proteins from endoplasmic reticulum (ER) and mediates the retro-translocation of proteins from ER lumen into cytosol (5–7). Derlin-1 expression is up-regulated by inducers of ER stress in yeast (8) and Caenorhabditis elegans (6) and suppresses ER stress-induced apoptosis in tumor cells (9). Almost all solid tumors encounter ER stress, such as hypoxia, and tumor growth depends on an intact unfolded protein response (10–13). Blocking the unfolded protein response also sensitizes cancers to chemotherapies (14).

Recently several lines of evidence have emerged and suggested that stress response proteins may leak to the surface of tumor cells and serve as molecular targets. A global profiling of the cell surface proteome of tumor cells has divulged a relative abundance of chaperone, heat shock, and glucose-regulated proteins (15). Hsp47, GRP78, and gp96, which are also restricted to the ER membrane, may be expressed independent of their chaperone properties and somehow escape the surveillance mechanism and end up on the tumor cell surface (16–19). Furthermore, Hsp47 and GRP78 were shown to be tumor-specific antigens for the targeting therapy (20–22).

In the present study, we showed that Derlin-1 was overexpressed on malignant cell surface. Polyclonal or monoclonal antibodies directed against Derlin-1 can target the tumor cell surface in vitro and in vivo and suppress tumor growth directly. This antibody-antigen system represents a novel therapeutic target that we hope will be considered for validation against tumors.

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Translational Relevance

Tumor targeting therapy is one of the most promising strategies for anticancer treatment. We elucidate herein that Derlin-1 can leak to the plasmalemma from the ER in tumor cells. Further more, strong membrane expression of Derlin-1 was detected in various types of human cancers tissues. Biodistribution analysis showed that Derlin-1 directed antibody specifically targeted to colon tumors in isogenic mice. Anti-Derlin-1-based treatment with polyclonal or monoclonal antibody could significantly suppress xenograft growth in isogenic mice. These preclinical data show that Derlin-1 protein is a functional molecular target expressed on the tumor cell surface and is a candidate therapeutic target that may be translated into clinical applications. The anti-Derlin-1 antibody may be developed to an effective anticancer drug for various types of tumor treatment.

Materials and Methods

Antibodies. Anti-Derlin-1 antibody was generated by immunizing rabbits or mice with peptides coupled to keyhole-limpet hemocyanin through an additional cysteine residue. The sequence of the immunizing peptide (p383) was (C)RHWNWGFPRLDQ (5), corresponding to the COOH-terminal peptide of the human Derlin-1 protein. Rabbit antibodies specific to the p383 peptide (anti-p383 antibody) were affinity purified from antiserum by Sepharose 4B conjugated to the p383 peptide. Three IgM monoclonal antibodies (8A2, 9F11, and 9F12) against Derlin-1 were generated and identified by ELISA against p383 peptide.

Tissue microarray and immunohistochemical staining. Cancerous tissues and matched normal samples were collected for tissue microarray from the Department of Pathology, Cancer Institute, Chinese Academy of Medical Science. Human tissues and engrafted tumors were formalin-fixed and paraffin-embedded for standard immunohistochemical staining and visualized by biotin streptavidin-peroxidase procedure. The primary antibodies included anti-p383 antibodies (10 μg/mL) and anti-Ki-67 antibody (diluted 1:100; Zymed Laboratories).

Cell surface immunofluorescent analysis. Dissociated cells were subjected to immunofluorescent analysis according to ref. 23. The primary antibodies were anti-p383 (10 μg/mL), mouse anti-α-tubulin (10 μg/mL; Sigma), or mouse anti-CEA (1:50; Abcam). The cells were visualized with Cy3-goat anti-rabbit IgG and FITC-goat anti-mouse IgG (diluted 1:400; Jackson ImmunoResearch Laboratories).

Preparation of apical or basolateral plasma membrane and cytoplasmic protein fractions for Western blotting. LS180 colon carcinoma cells were cultured in dishes until 90% confluent for the preparation of apical plasma or basolateral plasma membrane and cytoplasmic fractions according to ref. 24. Proteins of cellular fractions were then subjected to standard immunoblotting analysis with primary antibody of anti-p383 antibody or anti-Derlin1 antibody from Sigma.

Three-dimensional matrix culture. Briefly, a mixture of 1.5 mg/mL collagen I (BD Biosciences) and 1.0 × 10^5 LS180 cells/mL was incubated at 37°C for 36 h, and the cells were then released from the matrix by collagenase (Merck) for immunofluorescence analysis.

Tumor models. Female nu/nu mice (ages 4–6 weeks), obtained from the Jackson Laboratory, were used for production of ample, well-circumscribed s.c. tumors of LS180 cells in the right armpit of mice. Mice with burdened tumors of 5 to 10 mm in diameter were used for biodistribution analysis or γ-scintigraphic imaging.

Biodistribution analysis. The anti-p383 antibody or normal rabbit antibody were conjugated to 125I using iodogen as described in ref. 25. Biodistribution analysis was done as described previously (26). An experimental group and a control group, containing 12 tumor-bearing mice each, were anesthetized and injected via the tail vein with 125I-labeled anti-p383 or normal rabbit antibody (5 μg IgG; 12 μCi/μg), respectively. At each point of time, three mice of each group were anesthetized for the biodistribution analysis of the 125I antibody.

γ-Scintigraphic imaging. Six tumor-bearing mice were administered 100 μCi (80 μg) of the 125I-labeled anti-p383 antibody through the tail vein. For the control, four mice were simultaneously administered 0.8 mg cold anti-p383 antibody (without 125I labeling) or normal rabbit IgG. At different time points, the mice were imaged by γ-camera.

Anti-Derlin-1 antibody treatment of tumor-bearing mice. Tumor-bearing mice were size matched and divided into groups. The weights of mice were similar (~5% variation) within each treatment cohort. Treatments began 3 days post-injection of the tumor cells; two groups were treated with a 200 μg/mouse dose (~10 mg/kg, high dose) or a 40 μg/mouse dose (~2 mg/kg, low dose) of anti-p383 antibody, whereas two groups of control received corresponding doses of normal rabbit antibody or PBS alone. Three cohorts were also treated with three monoclonal antibodies (8A2, 9F11, and 9F12) of 200 μg/mouse.
respectively. A control group was treated with PBS. Therapeutic or control doses were systemically administered celiac in 200 μL PBS once daily for 5 days followed by twice weekly until treatment was completed. Tumor volumes were calculated according to the following formula: volume = length × width² / 2. Mice were sacrificed to measure tumor weight when treatment ended.

Statistical analysis and topologic analysis. Experimental results are expressed as mean ± SE of triplicate plates. Statistical significance was determined by Student’s t tests. Software used for topologic analysis of Derlin-1 included WoLF PSORT, SubLoc v1.0, and LOCSVMPSI.

Results

Derlin-1 is overexpressed on the malignant cell surface. Double immunofluorescence labeling was done with anti-Derlin-1 (anti-p383 antibody) and anti-α-tubulin antibody. The α-tubulin immunoreaction was conducted to confirm that the cytoplasm was accessible to antibodies only in cells fixed and permeabilized with Triton X-100 but not in fixed cells without permeabilization.

ER membrane-located Derlin-1 could be easily detected in the cytoplasm of permeabilized LS180 and other colon tumor cells (HCT116, LS174T, and SW480; Supplementary Fig. S1). Double immunofluorescence was further done with these cells after they were scraped from the dishes without permeabilization. Under confocal microscopy, the Cy3 staining of Derlin-1 adopted typical ring-shaped morphology around the periphery of impermeable cells (Fig. 1A; Supplementary Fig. S1). Simultaneously, α-tubulin was not detectable in these cells, showing that the cell membrane was intact and indeed impermeable to antibody. When normal rabbit IgG was used as primary antibody control in the immunofluorescence analysis, no positive signal was observed. Furthermore, the cell surface binding of anti-p383 antibody was inhibited in the presence of an excess (25 μg/mL) of p383 peptide (Fig. 1B) but was unaffected by another peptide of Derlin-1 COOH-terminal domain (p382, CRRADQNGGGGR, 25 μg/mL; Fig. 1C), which further showed the specific interaction of anti-p383 antibody with the COOH-terminal of Derlin-1 protein. Cell surface expression of Derlin-1 was repeated shown by flow cytometry in the colon tumor cell lines, as well as other tumor cells, including SKOV3 (an ovarian cancer cell line), MDA-MB-453 (a breast cancer cell line), Suit II (a pancreatic cancer cell line), H520 (a lung cancer cell line), and Yes2 (an esophageal cancer cell line; Supplementary Fig. S2). Figure 1D shows the percentage of Derlin-1 membrane expression cells in each tumor cell line according to flow cytometry analysis. Because Derlin-1 was seldom detected on Suit II cell surface, this cell was further permeabilized by Triton X-100 to act as positive control for cytoplasmic Derlin-1 and α-tubulin detection. In accordance with microscopy immunofluorescence analysis (Supplementary Fig. S1), strong cytoplasmic Derlin-1 and α-tubulin expression could be observed in most of the permeable cells. This again indicated the cell membrane was impermeable to antibody if the cell was not undergone permeabilizing treatment, for the α-tubulin reaction was really weak on nonpermeabilized cells. LS174T cells were transient transfected with myc-tagged Derlin-1 and were then subjected to double immunofluorescence staining with anti-p383 antibody (Cy3) or 9E10 anti-c-myc tag (FITC). The red and green signal could be colocalized on the surface of impermeable cells. LS174T cells, which were transient transfected with GFP-Derlin-1, were subjected to double immunofluorescence analysis and anti-p383 antibody signal could colocalized with GFP in the permeabilized cells (Supplementary Fig. S3). Together, the strong Derlin-1 immunoreactions on the nonpermeabilized cells were due to membrane location of Derlin-1 of these tumor cells.

The tumor cell surface expression of Derlin-1 protein was further shown by double immunofluorescence staining with anti-CEA. The Cy3 staining of Derlin-1 was colocalized with the
FITC staining of CEA, a common membrane protein of many adenocarcinoma cells (ref. 27; Fig. 1E).

Cell surface expression of Derlin-1 was induced by extracellular matrix in vitro. It has been established that Derlin-1 integrated in the ER membrane, adopting a four-pass transmembrane conformation, with its COOH terminus in the cytosol (6, 28), whereas topologic analysis predicts that Derlin-1 is a plasmalemma integrated protein, which spans the membrane 5 times with an extracellular COOH terminus (Fig. 2A). This prediction is consistent with our observations. Because there are several trypsin digestion sites in the COOH-terminal domain of Derlin-1, we digested the tumor cells with trypsin and then attempted to detect Derlin-1 with the anti-p383 antibody (Fig. 2B). In these digested but not permeabilized cells, Derlin-1 was undetectable.

However, cell surface expression of Derlin-1 could not be detected when immunofluorescence analysis was done with slide-attached cells (data not shown). We hypothesized that the inaccessibility of Derlin-1 protein to p383 antibody might due to the basolateral plasma membrane location of Derlin-1. To investigate this possibility, we prepared apical and basolateral membranes as well as cytoplasmic fractions of LS180 cells according to a silica microbead procedure. Protein of each fraction was subjected to Western blotting (Fig. 2C). In the cytoplasmic fraction, intensive expression of the 24 kDa Derlin-1 protein was detected, which represented the ER-resident protein.

Fig. 3. Expression of Derlin-1 is up-regulated in human cancer tissues. Left, Derlin-1 is overexpressed in six types of carcinomas by immunohistochemical staining on the tissue microarray. Cytoplasmic as well as plasmalemma expression of Derlin-1 is up-regulated in cancerous tissues. Right, statistical plots of Derlin-1 immunohistochemical staining in cytoplasm and membrane. Pancreas carcinomas, n = 87; normal pancreas tissue, n = 62. Hepatic carcinomas, n = 67; normal liver tissues, n = 60. Breast carcinomas, n = 64; normal breast tissues, n = 60. Lung adenocarcinomas, n = 19; normal lung tissues, n = 21. Colon adenocarcinomas, n = 55; normal colon tissues, n = 53. Esophageal squamous carcinomas, n = 52; normal esophagus tissues, n = 53. □, cancerous tissues; ■, normal tissues. The staining intensity was graded as three levels. Strong staining was leveled as 3, moderate staining as 2, and faint staining as 1. Immunohistochemical staining was scored based on positive cell percentage (tumor or matched normal cells) and staining intensity of these cells (score = staining level × positive cell percentage × 100). Both cytoplasmic and membrane immunostaining scores of all indicated types of cancer are significantly higher than the counterpart normal tissues.
Derlin-1 in tumor cells. There was a clear protein band of ~40 kDa in the basolateral plasma membrane fraction. This protein band may represent a homodimer of Derlin-1. Another anti-Derlin-1 antibody from Sigma was also used for Western blotting, and the result was similar with anti-p383 antibody (Supplementary Fig. S4). A dimer composed of GFP-tagged Derlin-1 with endogenous Derlin-1 has also been shown by Ye et al. (29). Although the 24 kDa form of Derlin-1 was also detected in the apical plasmalemma fraction, this form of Derlin-1 protein should located inside but not exposed outside the apical plasmalemma, for it could not be detected by immunofluorescence analysis. We considered that ventral membrane expression of Derlin-1 might be induced by interactions with the extracellular matrix, which was excreted by the tumor cells themselves. As expected, membrane expression of Derlin-1 was readily detected in the three-dimensional collagen I cultured SL180 cells (Fig. 2D).

Expression of Derlin-1 is up-regulated in cancerous tissues. Immunohistochemistry was done to validate the expression of Derlin-1 in cancerous and normal human tissues. Cytoplasmic Derlin-1 was significantly overexpressed in various cancers compared with matching normal tissues (Fig. 3). This coincides with the situation that tumors encounter a higher level of unfolded protein response stress (30). To further define the correlation of Derlin-1 expression and unfolded protein response activation, we treated colon tumor cells with tunicamycin or serum starvation to induce ER stress. Derlin-1 was up-regulated by these treatments as well as GPR78 (Supplementary Fig. S5), which is concordant with the result of Wang et al. (9). Membrane location of Derlin-1 was frequently observed in the cancerous tissues but was seldom observed in nonmalignant tissues (Fig. 3). Further analysis revealed that neither cytoplasmic nor membrane expression of Derlin-1 was correlated with cancer grade (histology) or tumor-node-metastasis stages in 87 pancreatic cancers and 62 normal pancreas tissues. Human normal tissues were also subjected to immunohistochemistry for Derlin-1 expression pattern analysis. Weakly cytoplasmic expression of Derlin-1 was observed in normal tissues, whereas membrane expression was seldom observed (Supplementary Fig. S6).

Malignant colon cells were isolated from colon tissue immediately after surgical removal for immunofluorescence analysis with confocal microscope (Supplementary Fig. S7A). In the intact and impermeable cells, Cy3-labeled Derlin-1 was readily colocalized on the cell surface with FITC-labeled CEA. In serial frozen sections of colon cancer tissues, robust expression of Derlin-1 on the plasma membrane could be partially overlaid with cell surface resident CEA (Supplementary Fig. S7B).

Derlin-1-directed antibodies selectively target tumors in an isogenic mouse model. Human Derlin-1 protein shares 98% identity with murine Derlin-1 and the COOH terminus of this protein is completely conserved between both species. As expected from this, the anti-p383 antibody can also recognize mouse Derlin-1 (Supplementary Fig. S8). Thus, tumor-bearing mice can be used as an isogenic model for in vivo targeting of Derlin-1. In biodistribution analysis, %ID/g and tumor/normal tissue ratios were analyzed at 24, 72, 120, and 168 h post-administration. The %ID/g in tumors increased, whereas the %ID/g in all normal organs and blood reduced with time. Seven days after injection, the %ID/g in all observed organs was obviously lower than that in tumors (Fig. 4A). Only the %ID/g in blood was ~26% higher than that in tumor, which might be due to the long half-life of rabbit antibody in mice.

![Image](Fig. 4. 125I-labeled anti-Derlin-1 antibody selectively targets to LS180 engrafted tumor in isogenic mice model. A, %ID/g at indicated time post-administration via the tail vein. B, tumor/normal tissue ratio (T/NT) at indicated time post-administration via the tail vein. C and D, γ-scintigraphic imaging of a LS180 tumor-bearing mouse. The autoradiographs were recorded at the indicated hours after injecting the 125I-labeled anti-p383 antibody into the tail vein (C). The radioactivity increasingly concentrated at the tumor location with time. The autoradiographs were recorded at the indicated hours after injecting the 125I-labeled normal rabbit antibody via the tail vein (D). The radioactivity did not increasingly concentrate in the tumor with time.)
Tumor/normal tissue ratio also indicated the tumor-specific immunotargeting of anti-p383 antibody in vivo, as these values continued to rise considerably over the 7 days (Fig. 4B). For control group, the %ID/g in all observed organs, as well as tumors, decreased with time. The tumor/normal tissue ratio of $^{125}$I-radiolabeled control IgG did not increase with time.

In γ-scintigraphic imaging, the autoradiographs were recorded at 24, 72, 120, and 168 h post-administration (Fig. 4C). Biodistribution visualization revealed the increasing accumulation of anti-p383 antibody in tumor. The tumor-targeting of $^{125}$I-labeled anti-p383 antibody could be inhibited by the competition with 10-fold excess of cold anti-p383 antibody (Fig. 4D) but not by normal rabbit IgG.

**Derlin-1 targeting antibodies suppress tumor growth in vivo.** Nude mice bearing LS180 colon tumor xenografts were treated with Derlin-1 targeting antibodies. The tumor growth was significantly suppressed by the treatment of anti-p383 antibody, 8A2, 9F11, or 9F12 monoclonal antibodies (Fig. 5A and B). The tumor burden was reduced by ~50% after 18 (both high and low doses of anti-p383 antibody) or 17 (monoclonal antibodies) days of s.c. injection of tumor cells. The organs, including heart, lung, liver, kidney, spleen, and enteron, were subjected to histologic analysis. No obvious systemic side effects were observed in the organs of treated mice (Supplementary Fig. S9). The average mouse weight, excluding the tumor weight, of the treated and control groups did not show significant difference when the treatment ended (Supplementary Fig. S10).

The overall tumor growth is determined by the balance of cell proliferation and programmed cell death; therefore, the cell proliferation-related protein Ki-67 was analyzed by immunohistochemistry and showed a significantly higher frequency of Ki-67 expression in tumors of the control group than in tumors of the anti-p383 antibody-treated groups (Fig. 5C). We did not observe different apoptotic level by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay or different microvascular density between these two groups of tumors (data not shown).

**Discussion**

For the targeting therapy, there exists an ongoing requirement to identify novel neoplastic cell-specific targets to develop tumor-targeting drugs. As we know, only ~20% of breast cancers respond to rhuMabHER2, because HER2 is overexpressed in only 25% to 30% of breast cancers (31, 32). Therefore, the targeting therapy calls for the suppression of more commonly overexpressed molecules in tumors. In the present study, tumor cell surface expression of Derlin-1 was validated by the Derlin-1 targeting antibody.
Surface expression of Derlin-1 was shown by immuno-fluorescence and flow cytometry analysis on non-permeabilized tumor cells as well as by the colocalization of Derlin-1 with CEA. Further study revealed that Derlin-1 might adopt a homodimer conformation in the lipid bilayer, with its COOH terminus residing outside the cell, and was only expressed in ventral membrane under condition of extracellular matrix induction. As basolateral membrane located molecule was inaccessible to antibody, this observation might explain why few published data revealed the surface expression of Derlin-1 on malignant cells and why we did not observe growth inhibition by anti-Derlin-1 antibody in *vivo*, although most of the immunoreaction studies are based on the COOH terminus of Derlin-1 to generate the anti-Derlin-1 antisera 

(5–7, 29, 33).

Immunohistochemistry revealed that overexpressed Derlin-1 was common in human cancers, including cancers of the pancreas, liver, breast, lung, colon, and esophagus. Simultaneously, membrane overexpressed Derlin-1 was frequently observed in these malignant tissues but seldom observed in normal tissues. In human tissues, the tumor cell surface expression of Derlin-1 was also detected by colocalization with CEA in immunofluorescence analysis of frozen colon cancerous tissue and tumor cells derived from fresh colon carcinoma tissues.

Biodistribution analysis and γ-scintigraphic imaging reveal that Derlin-1 antibody could rapidly and specifically home to tumors, although the anti-p383 antibody could react with endogenous mouse Derlin-1. Even 7 days after an i.v. injection, Derlin-1 antibody was retained in tumors with high concentration. This observation indicated that Derlin-1 was rarely or only sparsely expressed on the cell surface of normal organs and tissues of mice in accordance with the results of human tissues. Thus, we consider that the cell surface overexpression of Derlin-1 is tumor specific.

Finally, we tested the therapeutic properties of Derlin-1 targeting antibodies *in vivo*. The antitumor effects of the anti-p383 antibody (both 2 and 10 mg/kg antibody-treated groups) and the three monoclonal antibodies of Derlin-1 were similar, causing a reduction greater than 40% in tumor growth. The maximal rate of tumor growth suppression by Derlin-1 antibody-antigen system was closed to 50% in LS180 xenografts in *vivo* model. Anti-p383 antibody (2 mg/kg) should be the dosage close to the maximal effective dose. We did not observe any obvious systemic side effects even when the dosage was increased to 10 mg/kg. Therefore, the maximal tolerated dose should be higher than 10 mg/kg. We also found the therapeutic effect was apparently due to suppression of tumor cell proliferation but not tumor-related angiogenic inhibition by anti-Derlin-1 treatment, although overexpressed Derlin-1 contributes to tube formation of activated endothelium (34).

Taken together, our data show for the first time that Derlin-1 is expressed on the surface of tumor cells, which is induced by the extracellular matrix. Monoclonal antibodies of Derlin-1 shows effective antitumor activity against cell surface located Derlin-1.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


33. Lilley BN, Ploegh HL. Multienzyme complexes that link dislocation, ubiquitination, and extraction of misfolded proteins from the endoplasmic reticulum membrane. Proc Natl Acad Sci U S A 2006;102:14296–301.
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